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Microbial nitrogen transformations in earthworm burrows

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Abstract

Earthworms play an active role in soil nitrogen cycling. Past research has shown that earthworm castings are enriched in NO_3^- and NH_4^+ and show a high potential for microbial nitrification and denitrification. Little information is available on microbial populations and N transformations in the 1–2 mm thick soil lining of earthworm burrows (the drilosphere). We measured nitrifying bacterial populations, denitrifying bacterial populations, nitrification rates and denitrification rates of drilosphere and nondrilosphere soils. These measurements, in addition to measurements of NO_3^- concentration, NH_4^+ concentration, soluble organic-C, pH and water content, were performed on drilosphere material from laboratory microcosms inoculated with *Lumbricus terrestris* L. and on drilosphere material collected from earthworm burrows in long term no-till plots. The drilosphere soil was enriched in NO_3^-, NH_4^+ and soluble organic C and these soils had elevated populations of nitrifying and denitrifying bacteria relative to nondrilosphere soil. Drilosphere soil also had higher nitrification and denitrification rates. We postulate that earthworm-derived C and N deposited in the drilosphere facilitates the enrichment of N-transforming bacterial populations and that the elevated N-transformation rates results in an enrichment of NO_3^- in the earthworm burrow. This phenomenon has the potential for increased downward NO_3^- transport; however, the extent to which this potential is realized is not known. © 1999 Elsevier Science Ltd. All rights reserved.

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1. Introduction

Earthworms are dynamic members of the soil ecosystem. Earthworms ingest organic material and facilitate the redistribution of crop residues and organic matter throughout the soil profile (MacKay and Kladivko, 1985; Scheu, 1987a; Zhang and Hendrix, 1995). There have been numerous studies of the effects of earthworms on nutrient cycling. It has been observed that earthworm castings contain elevated amounts of NH_4, NO_3, Mg, K and P relative to bulk soil (Lunt and Jacobson, 1944; Parle, 1963; Gupta and Sakal, 1967; Syers et al., 1979; Tiwari et al., 1989). Studies of microbially-mediated N transformations associated with earthworm castings indicate elevated nitrification (Parle, 1963; Syers et al., 1979) and denitrification activities (Svensson et al., 1986; Elliott et al., 1990; Parkin and Berry, 1994). Daniel and Anderson (1992) reported elevated microbial respiration and bacterial counts in earthworm casts, yet no significant change in microbial biomass C.

An important result of earthworm activity is the creation of channels and pores throughout the soil volume. Earthworm burrows facilitate gas exchange and water movement. Earthworm burrows have also been implicated in the preferential flow of water and solutes (Ehlers, 1975; Zachmann et al., 1987; Edwards et al., 1988, 1989) and it has been reported that the walls of earthworm burrows are enriched in NO_3^- and labile-C (Syers and Springett, 1983) that may be transported by infiltrating water.

Despite the potential importance of earthworm burrows to the quantity and quality of infiltrating water, there have been few studies of the chemical characteristics of drilosphere (Lavelle, 1988; Binet and Trehen,

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1992; Stehouwer et al., 1993, 1994) and none investigating the nitrogen transformations and microbial populations associated with the soil lining earthworm burrows. The microenvironment associated with the walls of earthworm burrows may be substantially different from soil only a few millimeters away. Walls of the burrows of *Lumbricus terrestris* (L.) are smooth and cemented together with mucous secretions (Lavelle, 1988). The mucous secretions contain high concentrations of organic N and ammonium (Needham, 1957) and may serve as a substrate for fungi and bacteria (Edwards and Fletcher, 1988). Also, earthworm castings that are ejected in the burrow and subsequently pressed into the side of the burrow wall contain elevated amounts of nitrate and ammonium (Edwards and Lofty, 1980). In a study using ^{15}N-labeled rye residue, Binet and Trehen (1992) observed that the ^{15}N translocated from the surface applied residue was 3 times greater in the burrow wall than in the surrounding soil. These investigators also determined that most of the burrow-associated N was located in the first 2 mm of the burrow wall.

While it is clear that the soil material associated with earthworm burrows may provide a substantially different environment to soil microorganisms, we are unaware of studies conducted to ascertain the populations or activities of microorganisms responsible for major N transformations in the soil lining earthworm burrows. Our objective was to assess the populations and activities of N-transforming microbial communities the drilosphere in relation to nondrilosphere soil. These determinations were made on drilosphere material generated in laboratory microcosms by *L. terrestris* (L.) and in drilosphere material collected from earthworm burrows in the field.

2. Materials and methods

2.1. Laboratory experiments

Earthworm burrow material was generated in the laboratory by maintaining earthworms (*Lumbricus terrestris*) in plexiglass chambers with removable sides. The chambers were 30 cm tall, 40 cm wide by 5 cm deep and were filled with air dried soil (6 kg) that was packed to a bulk density of approximately 1.1 g cm^{-3}. The soil was a Clarion loam of glacial origin (fine-loamy, mixed, mesic Typic Hapludolls), having a texture of 46% sand, 34% silt and 20% clay and an average organic C content of 18.5 g C kg^{-1} soil. The soil was collected from a site that had a history of monoculture corn since 1977 (Berry and Karlen, 1993). Distilled water was added to each chamber to adjust the soil water content to 0.30 g g^{-1}. Four earthworms were added to each chamber and dried corn stover (5 g) was spread on the top of the soil to serve as a food source for the earthworms. Chambers were kept at 25°C, in the dark. Each week chambers were weighed to assess water loss and distilled water was added to maintain soil water content at 0.30 g g^{-1}. These incubation conditions are similar to those used by Bohlen and Edwards (1995) in their study of the influence of earthworms on nutrient transformations.

After 12 weeks, chambers were sampled by removing chamber side walls to expose the earthworm burrows. Drilosphere soil (burrow wall soil) was collected with a sterile spatula by carefully carving the inside 12 mm lining of the earthworm burrows. Nondrilosphere soil, at least 3 cm away from earthworm burrows, was also collected. Drilosphere and nondrilosphere soils from three replicate chambers were collected. Soil samples were immediately subsampled and analyzed for denitrification rate, denitrification potential, nitrification potential, denitrifying bacteria counts, nitrifying bacteria counts, water content, nitrate, ammonium, soluble C and total C.

2.2. Field sampling

Burrow and nonburrow soil was collected from no-till plots in Ankeny, IA. The field plots had a history of 17 yr of continuous corn with no-tillage management (Berry and Karlen, 1993) and were sampled in early October before corn harvest. As in the laboratory experiments the soil type was a Clarion loam. Past studies indicated that in the fall this site supported *L. terrestris* populations ranging from 20.6–36 individuals m^{-2} (Berry and Karlen, 1993). Burrow material was collected by excavating small soil blocks (ca. $5 \times 5 \times 8$ cm deep) that contained a surface earthworm burrow; however, it could not be assured that the burrows sampled were exclusively from *L. terrestris*. Soil blocks were gently broken apart to expose the earthworm burrow and the 12 mm layer of burrow wall soil was collected with a sterile spatula. Soil not associated with the earthworm burrow was also collected from each monolith. Soils from 8 to 12 burrows were composited for each plot and a composite nonburrow soil sample was also collected from each plot. Three replicate plots were sampled. Soil samples were analyzed for chemical composition, denitrification, nitrification and bacterial populations.

2.3. Analyses

Samples for NO_3 and NH_4^+ (approximately 5 g moist soil) were extracted with 20 ml 2 M KCl by shaking for 2 h at 25°C and then filtered. NO_3 ($NO_3 + NO_2$) was determined by the cadmium reduction method and NH_4^+ determined by the indophenol blue method (Keeney and Nelson, 1982) using an

Table 1
Physical and chemical properties of drilosphere and nondrilosphere soil from laboratory experiment and field sampling. Values in parenthesis are %Coefficient of Variation

Sample location	Water content (g g^{-1})	Nitrate (μg N g^{-1})	Ammonium (μg N g^{-1})	Soluble organic C (μg C g^{-1})	pH
Laboratory					
Burrow	0.30 (10.0)	26.6 (11.1)	13.0 (29.8)	258 (3.1)	6.35
Nonburrow	0.31 (12.9)	18.8 (12.8)	10.5 (12.6)	219 (17.8)	5.72
Probability	0.598	0.003	0.490	0.163	–
Field					
Burrow	0.18 (11.1)	9.22 (9.5)	< 0.50	136 (4.0)	6.14 (0.17)
Nonburrow	0.13 (2.5)	5.86 (15.4)	< 0.50	104 (10.8)	4.78 (0.12)
Probability	0.034	0.001	–	0.077	0.043

automated chemistry unit (Lachat Instruments, Milwaukee, WI). NO_3 and NH_4^+ results are expressed on a dry weight basis. Water content was determined gravimetrically, by oven drying 5 g subsamples at 105°C. pH was measured on 1:1 soil:water extracts using a glass electrode.

Soluble organic carbon determinations were performed by shaking 5 g moist soil with 50 ml 0.5 M K_2SO_4 for 30 min followed by centrifugation and filtration. The filtered extracts were acidified with 1 ml concentrated H_2PO_4 and sparged for 90 s with N_2 to remove carbonates. Soluble organic carbon was determined using a Dohrrman carbon analyzer (Rosemont Analytical, La Habra, CA)

Denitrification rates were determined using the acetylene block method (Yoshinari et al., 1977). Soil (5 g fresh material) was placed in 26 ml test tubes. The tubes were capped with rubber bungs leaving an air headspace and 1 ml C_2H_2 added to the headspace of each tube. Denitrification rates were calculated from N_2O production over 16 h at 24°C. Nitrous oxide was measured on a gas chromatograph equipped with an electron capture detector and using a modification of an automated method for sampling and analysis (Parkin, 1985).

Denitrification potential of soil was determined in anaerobic incubations. Five g fresh soil was placed in a 26 ml test tube and 5 ml of an aqueous 1 mM glucose, 10 mM NO_3^- solution were added. The test tubes were stoppered with rubber bungs and the headspace of each tube made anaerobic by flushing with He. Acetylene (1 ml) was added to the headspace of the tube and denitrification rates determined by monitoring N_2O production every 2 h during a 6 h incubation at 24°C.

Nitrification rates were determined by monitoring the increase in $NO_3^- + NO_2^-$ from 10 g of fresh soil incubated in 26 ml test tubes at 24°C for 48 h (Lensi et al., 1986). Rates of nitrification are expressed on a dry weight basis.

Numbers of nitrifying bacteria and denitrifying bacteria were determined by the most probable number technique (Alexander, 1982). Nitrifying bacteria were enumerated using the media of Schmidt and Belser (1982) to determine ammonium oxidizers and nitrite oxidizers. Denitrifying bacteria were enumerated using the procedure of Tiedje (1982). All enumerations were performed using 5 tube MPNs and 10-fold dilutions over the range of 10^4 to 10^8. Bacterial numbers are expressed on a g dry soil basis.

2.4. Statistical analyses

Comparisons of burrow with nonburrow soils were performed using a *t*-test on untransformed data. We considered differences to be significant at a probability level of 10%. Our judgement is based on the lack of power associated with the statistical test employed (only three replications were available) and our desire to decrease the probability of committing a type II error while only moderately affecting the type I error rate.

3. Results and discussion

The drilosphere soil (1–2 mm thick lining the burrow wall) of both the laboratory and field burrows was significantly higher in nitrate than soil not associated with earthworm burrows (Table 1). There was a trend of higher NH_4^+ in the drilosphere of the laboratory-derived burrows; however, concentrations were not significantly different than nondrilosphere soil. Ammonium was not detected in the field soils. Soluble organic carbon concentrations of both the laboratory and field drilosphere tended to be higher than nondrilosphere soil, but a significant difference was only noted for the field soils. Our observations of elevated nitrate concentrations in the drilosphere soils are consistent with elevated inorganic N associated with the excreta of earthworms reported in the literature by Parle (1963) and Syers and Springett (1984). The fact that we did not observe elevated ammonium concentrations could be due to temporal dynamics associated with N transformations in the burrow wall. Syers et al.

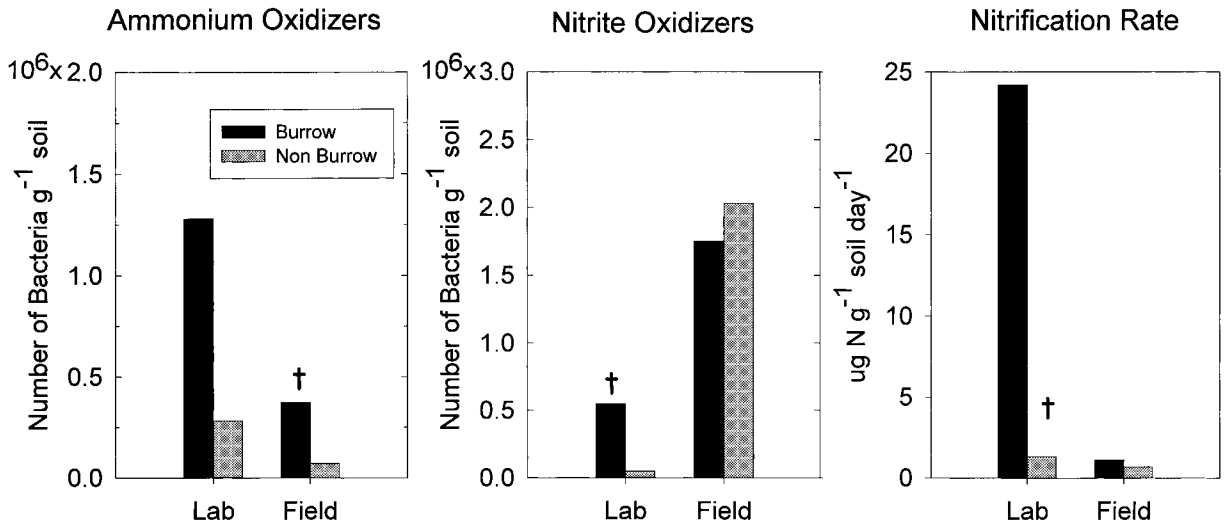

Fig. 1. Nitrifying bacterial populations and nitrification rates of burrow and nonburrow soil collected from laboratory-derived and field earthworm burrows. Significance differences between burrow and nonburrow soil at $P < 0.10$ are indicated by †.

(1979) reported seasonal variations of nitrate and ammonium concentrations in earthworm casts. Short term fluctuations in nitrate and ammonium have also been observed. Freshly deposited casts are initially high in ammonium, but within 2 weeks ammonium contents decline with a corresponding increase in nitrate concentration (Parle, 1963; Parkin and Berry, 1994).

The pH of burrow wall soil was higher than nonburrow soil. For the laboratory experiments, pH was measured only on a single sample but the field experiments indicate that the higher pH associated with the drilosphere is highly significant. The typical pH for Clarion soils ranges from slightly acid to neutral (US Department of Agriculture, 1981). The fact that the pH values measured in our study tended to be lower could be because the fields were under continuous corn with urea ammonium nitrate fertilizer added each year at a rate of 168 kg N ha^{-1}. This N fertility regime coupled with a lack of lime addition could have resulted in the lower pH values.

In these laboratory experiments, the water contents of burrow and nonburrow soil were not different, however, in the field, the drilosphere soil was significantly wetter than the bulk soil. It should be noted that for all the variables measured the comparisons are made on a gravimetric basis. We could not measure the bulk density of the drilosphere, however, if one assumes that the bulk density of the soil lining the burrow wall is greater than the nonburrow soil, then on a volumetric basis the differences between the burrow and nonburrow soil become greater.

The elevated NO_3 concentrations of the drilosphere soil are consistent with observations of elevated nitrifiying bacterial populations (Fig. 1). In the laboratory-derived material, the population of nitrite oxidizing bacteria was significantly higher in burrow soil. There was a trend of greater numbers of ammonium oxidizing bacteria associated with burrow walls, but this difference was not significant. In the field the drilosphere soil had a significantly higher ammonium oxidizing bacterial population than the nonburrow soil, while the populations of nitrite oxidizing bacteria were not significantly different. The nitrification rate associated with laboratory-derived burrow soil was nearly 20 times higher than nonburrow soil, but in field soils no significant differences were observed in nitrification rates (Fig. 1). High populations of both ammonium and nitrite oxidizing bacteria were observed in the field soils, thus it is likely that the ammonium availability was limiting nitrifying activity in these samples.

It is interesting that despite the higher nitrification activity and subsequent H^+ production associated with burrow material, the pH of the burrow wall material was significantly higher than surrounding soil. An explanation for this discrepancy could be due to the fact that many species of earthworms, including *L. terrestris*, contain calciferous glands that secrete calcium carbonate (Darwin, 1883; Lee, 1985). It has been proposed that calcium carbonate excretion may serve to regulate the pH of the earthworm gut (Needham, 1957). It is also possible, in the field experiments, that $CaCO_3$ is directly transported from the calcareous subsoil to the burrow lining by earthworm activity. Regardless of the precise mechanism, it seems likely that the earthworms may play an active role in pH regulation of the drilosphere and that this regulation may be necessitated by the high nitrification activity associated with earthworm casts.

Numbers of denitrifying bacteria were significantly

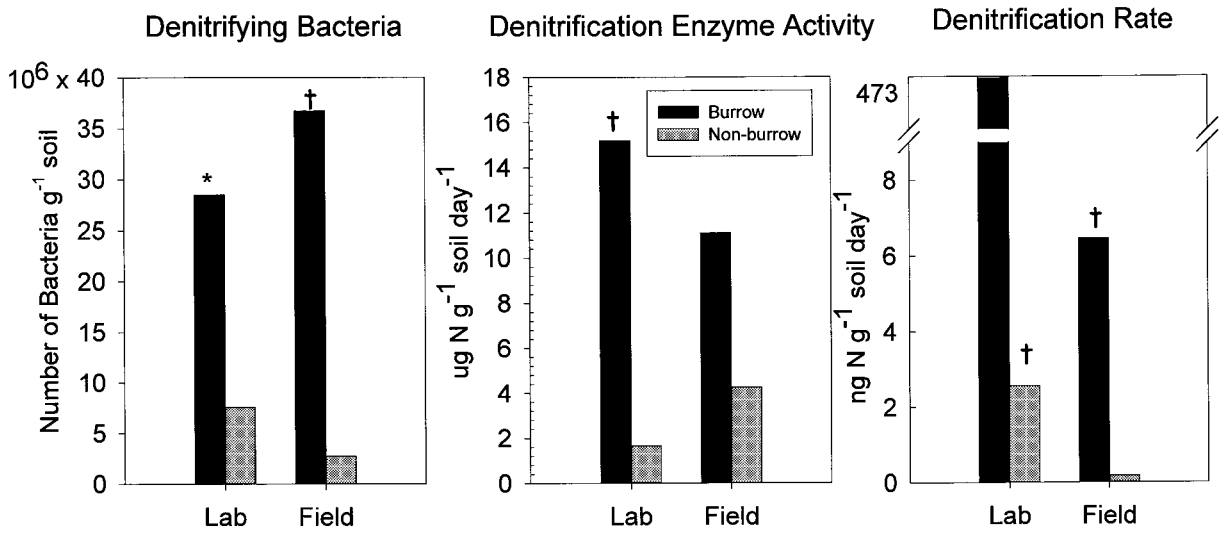

Fig. 2. Denitrifying bacterial populations, denitrification enzyme activity and denitrification rates of burrow and nonburrow soil collected from laboratory-derived and field earthworm burrows. Significance differences between burrow and nonburrow soil at $P < 0.10$ are indicated by †. Single asterisk indicates significant difference, $P < 0.05$.

higher in burrow soils as compared to nonburrow soils (Fig. 2). Denitrification enzyme activity, as determined in anaerobic incubations with added glucose and nitrate, was also higher in the drilosphere soils. Estimates of natural denitrification rates, determined by incubating soil in tubes with an air headspace and no amendments, were lower than denitrification enzyme activity measurements. In both the field and laboratory soil materials, there was a trend of higher denitrification rate in burrow wall material as compared to nonburrow material. In relation to N cycling, it is important to point out that denitrification rates are approximately 1000 times lower than nitrification rates. This would indicate that nitrate formed from nitrification has the potential to persist in the burrow, as denitrification would not contribute to rapid loss.

There have been mixed reports on the effects of earthworms on soil microorganisms. Elevated soil respiration, presumably due to soil microorganisms, has been reported in the presence of earthworms, yet decreased soil microbial biomass has been observed (Daniel and Anderson, 1992; Bohlen and Edwards, 1995). Earthworm castings have also shown increased microbial respiration (Parle, 1963; Scheu, 1987b) and increased bacterial counts (Satchell 1983; Daniel and Anderson, 1992). Despite the contradictory information in the literature concerning the influences of earthworms on microbial populations, our data indicate elevated populations of nitrifying bacteria and denitrifying bacteria associated with the drilosphere soil. The primary determinants responsible for these increased microbial populations are not precisely known, however, several possibilities exist. In the case of nitrifying bacteria, it is known that earthworms excrete ammoniacal compounds including NH_4^+, urea, allantoin and uric acid (Lee, 1985). A continuous supply of NH_4^+, coupled with the pH buffering afforded by the $CaCO_3$ excreted by some earthworm species would be advantageous to the development of nitrifying bacterial populations in the drilosphere. Denitrifying bacteria would find the elevated soil moisture contents, the deposition of earthworm-derived organic carbon such as mucoproteins and the compacted sides of the burrow, an advantageous environment.

Our results are consistent with other studies of the influence of earthworms on microbial nitrogen transformations. Earthworm casts are enriched in NO_3^- and NH_4^+ and have been observed to have elevated nitrification and denitrification activity (Svensson et al., 1986; Elliott et al., 1990; Parkin and Berry, 1994). It is not surprising to observe similar characteristics within the drilosphere, since earthworm excreta is often pressed into the burrow walls (Lee, 1985).

A critical issue regarding earthworm burrow chemistry and microbiology, is their effect on the quality of infiltrating water. Deposition of ammoniacal N, in the form of mucoproteins, urea and NH_4 can be substantial. Krishnamoorthy (1985) estimated N excretion rates of earthworm mucoprotein-N to be 21.3 μg N g earthworm^{-1} d^{-1} in a grassland system. In experiments using 15-N labeled ryegrass, Binet and Trehen (1992) measured N output from *L. terrestris* to the soil to be 76 μg N g earthworm^{-1} d^{-1}, with 30% of this deposition occurring in the burrow (23 μg N g earthworm^{-1} d^{-1}). The drilosphere nitrification rates of the laboratory incubations of our study (24.6 μg N g drilosphere soil^{-1} d^{-1}) indicates that ammonium would rapidly be

converted to NO_3^-. Thus, a potential exists for enhanced NO_3^- contamination of groundwater through macropore flow. For example, using the N deposition rates of Binet and Trehen, we estimate that for a 120 d period from June–October that the activities of a single *L. terrestris* will result in the deposition of 13.8 mg NO_3^- in the drilosphere of its burrow (assuming an earthworm weight of 5 g). At a density of 50 *Lumbricus* m^{-2}, this presents the potential for the leaching of 6.9 kg N ha^{-1}. However, data of Edwards et al. (1990) indicate that this potential for NO_3^- transport may not be fully expressed. Earthworm burrows (>0.5 mm) were instrumented with collection devices and water was collected for 12 rain storm events over the period June–October, 1987 (Edwards et al., 1990). From NO_3^- concentrations in the burrow water and from areal burrow estimates (205 burrows m^{-2}), these investigators estimate that only 0.711 kg N ha^{-1} was transported through earthworm burrows.

Denitrification may account for part the discrepancy between our estimates of NO_3^- available for macropore transport (6.9 kg N ha^{-1}) and the measurements of Edwards and coworkers. Using estimates of denitrification from our laboratory studies, we calculated that over 120 d NO_3^-–N loss in the drilosphere from denitrification to be 5.5 kg N ha^{-1} (assuming a denitrification rate of 0.47 μg N g drilosphere $soil^{-1}$ d^{-1}, a 2 mm thick drilosphere of burrows 5 cm dia and 90 cm long burrows, 205 burrows m^{-2} and burrow soil density = 1.2 g cm^{-3}).

Edwards et al. (1989) acknowledge that many of the burrows they sampled appeared to be abandoned and did not exhibit evidence of recent earthworm activity, thus one would expect lower N deposition and nitrification activity. However, it may also be likely that the laboratory-derived estimates of N deposition reported by Binet and Trehen (1992) may overestimate actual deposition of N in the field. We observed nitrification rates of field drilosphere soil to be only 5% of our laboratory-derived burrow material. Finally, a number of other factors may influence NO_3^- leaching from the drilosphere including drilosphere surface area and water contact time. While our study was not intended to provide a definitive description of the role of earthworm burrows in NO_3^- transport, this study does show that differences in N cycling may exist in the drilosphere. It is clear that earthworm burrows present a microenvironment different from surrounding soil and this environment supports elevated populations of N-transforming bacteria.

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